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AIC-138

PARTIAL PURIFICATION AND PROPERTIES OF TOMATIN, AN ANTIBIOTIC AGENT FROM THE TOMATO PLANT

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Introduction: Tomatin, an antibiotic agent that is present in the cultivated tomato plant (*Lycopersicon esculentum*) is capable of inhibiting the soil-borne fungus (*Fusarium oxysporum* f. *lycopersici*) that causes tomato wilt, an economically serious disease of this important food crop. Moreover, it has been found that the antibiotic effectiveness of tomatin is not limited to this one organism; it inhibits to an even greater extent, cultures of certain human pathogenic fungi and bacteria.

The results of these preliminary investigations point to two interesting possibilities: (A) that tomatin may be responsible, either wholly or in part, for the resistance offered by certain tomato varieties to Fusarium wilt; and (B) that because of its marked inhibitory action upon several important human pathogens (particularly the fungi), tomatin may be of therapeutic value in the treatment of human infections that are caused by these organisms.

The academic and practical significance of the possibilities that have been mentioned have encouraged us to make concerted efforts to isolate tomatin, to determine its structure, its origin, action and fate in the tomato plant, and its toxicity to animals and humans, and to extend the investigation of its antibiotic spectrum to the largest practicable number of different pathogenic organisms. The major part of this program remains to be accomplished. The results reported here are based upon observations made with partially purified, but still impure preparations of tomatin and accordingly, the interpretation of many of the results must be regarded as tentative.

Procedure for obtaining tomatin preparations: Tomatin is present in highest concentration in tomato leaves, to a lesser extent in the roots, and to the least extent in the stems and fruit. In the laboratory preparation of tomatin it is preferable to use the leaves of the Red Currant tomato plant (*Lycopersicon pimpinellifolium*) a Peruvian variety that bears currant-sized fruit, since this variety is the richest source of tomatin so far discovered. For practical preparative purposes, however, whole plants of any commercial variety may be used as the source of tomatin. Depending upon its accessibility, the plant material may be processed either in the fresh condition or after drying and grinding. A typical experiment, illustrating the procedure used for processing dried, whole tomato plants for tomatin and the yields that are to be expected, is described below. In discussing the results of this experiment and others, tomatin activity is expressed in arbitrary units, which are estimated by means of the familiar cylinder-plate technique, *Fusarium oxysporum* f. *lycopersici* being used as the test organism and a sterile tomatin solution, prepared from tomato leaves and stored in sealed ampoules, being used as the reference standard. A solution containing one unit of tomatin per ml. will produce an inhibition zone of 18.5 mm. on the standard assay plate.

Two hundred grams of dried tomato plant, estimated to contain 4400 units of tomatin activity, were extracted with four successive 1 liter portions of hot methanol. The clear, green extract was concentrated to dryness *in vacuo* and the residue was thoroughly extracted, portionwise, with boiling water to yield approximately 500 ml. of clear, yellow solution. Upon evaporation to dryness *in vacuo* this extract yielded 21 g. of crude tomatin containing approximately 4000 units.

These results indicate that a tomatin preparation which is nearly ten times as potent as the starting material can be obtained quite simply without much loss of activity.

When fresh plant material is to be processed, it is ground and pressed, and the juice is autoclaved at 120°C. for approximately one hour. The inactive precipitate which forms is separated; the clear extract is concentrated to dryness; and the residue is processed exactly as indicated above. In this manner tomatin preparations having the same potency as those obtained by processing dried material are obtained, but the yields are somewhat lower since the separation of the active juice from the fresh plant residue is inefficient unless excessive amounts of water are used.

Crude tomatin preparations contain approximately 0.2 units of tomatin activity per mg.. In numerous attempts to increase the potency of such preparations, fractions have been isolated which have potencies of 1 to 2 units per mg.. Unfortunately, these procedures have resulted in such prohibitive losses in total activity that the procedures so far utilized have been temporarily abandoned as impractical.

It is obvious that potencies in the range of 1 to 2 units per mg. appear insignificant when tomatin is compared with certain other antibiotic agents. As will be apparent later, however, the arbitrary unit (based on *Fusarium* as the test organism) that is used for the evaluation of tomatin attributes deceptively low antibiotic activity to tomatin when it is compared with other antibiotic agents. This is due in large part to the fact that, of all of the organisms so far tested, the Fusarium species are by far the least sensitive to the inhibitory action of tomatin. Nevertheless, we have adhered to the use of *Fusarium oxysporum* f. *lycopersici* as the test organism, because it is the activity of tomatin preparations toward various Fusarium species which will be of greatest significance in the study of wilt resistance in plants.

Physical and chemical properties of crude tomatin preparations: In aqueous solution (pH 5.5) in sealed ampoules, crude tomatin is able to withstand a temperature of 120°C. for at least 5 hours without detectable loss of antibiotic activity. Tomatin is dialyzable. Based on the study of crude preparations tomatin is soluble in water at pH values below neutrality, is very soluble in absolute methanol, and exhibits progressively decreased solubility in ethanol, isopropanol and butanol. Tomatin is insoluble in all of the other organic solvents that have been tried including aromatic and aliphatic hydrocarbons, ethers, esters and ketones, chloroform and dioxane. The antibiotic agent is adsorbed from slightly acid aqueous solution on charcoal (Norit A) and on alumina. Tomatin can be eluted from charcoal by the use of acid aqueous methanol.

Under certain conditions tomatin is precipitated, or is adsorbed on material that is precipitated from crude aqueous solutions when the solution is adjusted to pH values above neutrality. Although much inert material is eliminated in this manner, the loss of total tomatin activity by precipitation at alkaline pH values makes this method impractical for purification.

One preparation, having a potency of 1.2 tomatin units per mg., has been analyzed. It is realized that the preparation analyzed was quite impure. However, no sulfur was detected and a nitrogen value of less than 1 percent was obtained.

The most highly active preparations of tomatin that have been obtained contain sufficient color in both water and alcohol solutions to prevent measurement of optical activity. Crude tomatin preparations exhibit a strongly positive orcinol test, indicating the presence of carbohydrate. Phenolic tests and the ninhydrin test are negative.

Effect of tomatin on several microorganisms: The effects of tomatin upon a considerable number of microorganisms are given in Table 1, 2 and 3. It will be observed that the organisms have been divided into three groups depending upon whether they are completely inhibited, partially inhibited, or unaffected by the presence of 1 unit of tomatin per ml. in the culture medium. These results constitute only a rough screening test for the relative sensitivity of the organisms to tomatin and it is likely that in many cases complete or partial inhibition of growth would have occurred had far less tomatin been used. For instance, in the case of Trichophyton mentagrophytes, one of the organisms of the ringworm group affecting man, complete inhibition can be effected with as little as 0.2 unit tomatin per ml. of medium; in the case of Blastomyces dermatitidis, the organism associated with North American Blastomycosis, complete inhibition is attained at a tomatin level of 0.05 unit per ml. Conversely, it is possible that inhibition of some of the organisms that appear to be resistant to the action of tomatin might be accomplished by the use of higher tomatin concentrations.

It is evident from these results that tomatin is highly effective against cultures of the human dermatophytic fungi and against several of the fungi and yeast-like forms that cause internal disease. It is slightly effective under the conditions employed against the test species of bacteria, the fungi Fusarium, Penicillium, Aspergillus, Sporotrichum and Monosporium and the plant pathogenic Actinomyces. It is without significant effect upon the human pathogenic Actinomyces, and the fungi responsible for the Chromoblastomycoses.

It should be emphasized that the results presented in Tables 1, 2 and 3 are indicative of the inhibitory action of tomatin primarily upon the vegetative growth of the organisms tested. It is not feasible by the technique employed to observe accurately the fungicidal or bactericidal effects of tomatin. By the use of suitable methods, however it has been possible to demonstrate that tomatin is definitely fungicidal for spores of Fusarium oxysporum f. lycopersici even though it exerts only partial inhibition toward the vegetative proliferation of this same organism.

Toxicity tests: Preliminary toxicity tests, upon guinea pigs with a saline solution of a tomatin preparation containing 1.2 units per mg., have indicated that animals can tolerate the parenteral administration of a much as 10 mg. of such a preparation without fatal consequences, although unfavorable reactions are produced. The intravenous, intraperitoneal or subcutaneous injection of a solution containing 1 mg. of crude tomatin induced no notable reaction. However, the intravenous injection of 10 mg. into each of two animals produced immediate symptoms of distress and swelling of the injected legs. The animals recovered from the systemic reaction within 24 hours, but the injected legs remained swollen. Both animals continued to gain weight, and one recovered completely within 4 days; the second animal suffered necrosis of the injected leg. The intraperitoneal injection of 10 mg. of the tomatin preparation produced immediate and severe distress accompanied by a tensing of the abdomen. These symptoms subsided in 24 hours, and recovery was apparently complete. The subcutaneous injection of 10 mg. of the tomatin preparation induced a delayed systemic reaction from which the animal recovered within 24 hours. However, the cutaneous tissue at the site of the injection formed a persistent ulcer which was not healed completely 10 days after injection.

The presence of tomatin-like substances in other plants: Extracts of plants other than the tomato have been found to inhibit cultures of *Fusarium*. Tomatin-like activity has been demonstrated in the autoclaved juice of the following plants: Sweet-potato plants (*Ipomoea botatas*) including the roots, potato tops (*Solanum tuberosum*) but not the tubers, pepper (*Capsicum frutescens*) plants and cabbage (*Brassica oleracea* var *capitata*) leaves. It appears to be absent in lettuce (*Lactuca sativa*), jimson weed (*Datura stramonium*), white clover (*Trifolium repens*), dandelion (*Raraxacum offininale*) and lima beans (*Phaseolus lunatus*).

Significance of preliminary results: The antibiotic, and particularly the fungistatic properties of tomatin are extremely interesting. Tomatin is by no means the only antibiotic agent that possesses fungistatic properties but most, if not all, of the known antibiotic agents are limited in their therapeutic usefulness because of their high toxicity. In view of the somewhat encouraging results of tests for toxicity reported here, and the marked *in vitro* effectiveness of tomatin against numerous pathogenic fungi, a complete investigation of the *in vivo* activity of this antibiotic agent is warranted and is in progress.

From the evidence presented it is obvious that tomatin, in its action toward microorganisms, is not highly specific, Likewise, it appears that this lack of specificity may also be characteristic of the substances responsible for the tomatin-like activity of the juices of certain other plants. These results may be of fundamental significance in the investigation of the biochemical factors responsible for disease resistance in plants.

Summary: Directions for obtaining preparations of tomatin from tomato leaves, together with preliminary information concerning the chemical, physical and toxicological properties of tomatin preparations are presented. Tomatin has been found to be highly effective *in vitro* in inhibiting the human dermatophytic fungi, including species of *Trichophyton*, *Epidermophyton*, *Microsporum* and *Achorion*, and several of the fungi and yeast-like forms that cause internal disease, including species of *Monilia*, *Cryptococcus*, *Debaryomyces*, *Blastomyces*, *Coccidioides*, and *Histoplasma*. It is slightly effective against both gram positive and gram negative bacteria, species of the fungi *Fusarium*, *Penicillium*, *Aspergillus*, *Sporotrichum*, and *Monosproium* and the plant pathogenic *Actinomyces*. It is without effect upon the human pathogenic *Actinomyces* and the fungi responsible for the Chromoblastomycoses. The presence of tomatin-like substances in certain plants other than the tomato is reported.

TABLE I

Organisms Whose Growth is Completely Inhibited by the Presence of 1 unit Tomatin per ml. of Culture Medium

Organism	Disease and Remarks
Candida (Monilia) albicans	Moniliasis (Thrush, Bronchomycosis)
Cryptococcus neoformans	Cryptococcosis (European Blastomycosis, Torulosis)
Debaryomyces histolytica	
Trichophyton mentagrophytes	The Dermatomycoses (Ringworm, Athlete's Foot, etc.)
Trichophyton interdigitale	
Trichophyton rubrum	
Trichophyton gypseum	
Epidermophyton floccosum	
Microsporum audouini	
Achorion gypseum	
Achorion schoenleinii	
Blastomyces dermatitidis	North American Blastomycosis
Coccidioides immitis	Coccidioidomycosis
Histoplasma capsulatum	Histoplasmosis

TABLE II

Organisms Whose Growth is Partially Inhibited by the Presence of 1 unit Tomatin per ml. of Culture Medium

Organisms	Disease and Remarks
Fusarium oxysporum f. lycopersici	Tomato wilt
Fusarium oxysporum f. pisi	Pea wilt
Fusarium oxysporum f. conglutinans	Cabbage yellows
Fusarium oxysporum f. lini	Flax wilt
Actinomyces scabies	Potato scab
Sporotrichum schenckii	Sporotrichosis
Monosporium apiospermum	Maduromycosis (Madura Foot, Mycetoma)
Aspergillus niger	Rarely pathogenic
Aspergillus clavatus	
Penicillium notatum	
Staphylococcus aureus	Gram positive
Bacillus cereus	Gram positive
Bacillus mycoides	Gram positive
Bacillus subtilis	Gram positive
Escherichia coli	Gram negative

TABLE III

Organisms That are Unaffected by the Presence of 1 unit Tomatin per ml. of Culture Medium

Organism	Disease and Remarks
Actinomyces (Nocardia) asteroides	Actinomycosis (Lumpy Jaw)
Actinomyces hominis	
Phialophora verrucosa	Chromoblastomycosis
Hormodendrum pedrosoi	

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